Current Biology 16, 1672–1677, August 22, 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.06.059

Report

Biphasic Regulation of Mitochondrial Ca^{2+} Uptake by Cytosolic Ca^{2+} Concentration

Ben Moreau, Charmaine Nelson, and Anant B. Parekh*
Department of Physiology, Anatomy, and Genetics
Oxford University
Parks Road, Oxford OX1 3PT
United Kingdom

Summary

A rise in cytosolic Ca^{2+} concentration is used as a key activation signal in virtually all animal cells, where it triggers a range of responses including neurotransmitter release, muscle contraction, and cell growth and proliferation [1]. During intracellular Ca^{2+} signaling, mitochondria rapidly take up significant amounts of Ca^{2+} from the cytosol, and this stimulates energy production, alters the spatial and temporal profile of the intracellular Ca^{2+} signal, and triggers cell death [2–10]. Mitochondrial Ca^{2+} uptake occurs via a ruthenium-red-sensitive uniporter channel found in the inner membrane [11]. In spite of its critical importance, little is known about how the uniporter is regulated. Here, we report that the mitochondrial Ca^{2+} uniporter is gated by cytosolic Ca^{2+}. Ca^{2+} uptake into mitochondria is a Ca^{2+}-activated process with a requirement for functional calmodulin. However, cytosolic Ca^{2+} subsequently inactivates the uniporter, preventing further Ca^{2+} uptake. The uptake pathway and the inactivation process have relatively low Ca^{2+} affinities of approximately 10–20 μM. However, numerous mitochondria are within 20–100 nm of the endoplasmic reticulum, thereby enabling rapid and efficient transmission of Ca^{2+} release into adjacent mitochondria by $InsP_3$ receptors on the endoplasmic reticulum. Hence, biphasic control of mitochondrial Ca^{2+} uptake by Ca^{2+} provides a novel basis for complex physiological patterns of intracellular Ca^{2+} signaling.

Results and Discussion

In spite of the importance of the mitochondrial uniporter in shaping intracellular Ca^{2+} signals and subsequent downstream responses, little is known about how it is regulated.

To investigate this, we measured Ca^{2+} concentration within the mitochondrial matrix by using the Ca^{2+} indicator dye rhod-2 in permeabilized RBL-1 cells. Application of 10 μM Ca^{2+} to the cytosol resulted in a rapid rise in mitochondrial Ca^{2+} concentration (Figure 1A). After removal of cytosolic Ca^{2+}, mitochondrial Ca^{2+} slowly returned to prestimulation levels with a half-time of approximately 300 s, consistent with previous reports [12, 13]. Several pieces of evidence demonstrate that the rhod-2 signal emanates from dye compartmentalized within the mitochondrial matrix. First, depolarization of the mitochondrial membrane potential with the protonophore FCCP prevented rhod-2 fluorescence from increasing after application of a range of cytoplasmic Ca^{2+} concentrations (10–100 μM Ca^{2+}, Figure 1B). Conversely, the slow decline of the rhod-2 signal (as in Figure 1A) was accelerated by application of FCCP (data not shown). Second, the rise in rhod-2 fluorescence upon exposure to 10–25 μM cytosolic Ca^{2+} was completely blocked by inhibition of the mitochondrial uniporter with ruthenium red (Figure 1C). The $InsP_3$ receptor antagonist, heparin, had no effect on the rise in rhod-2 fluorescence when cytosolic Ca^{2+} concentration was increased, ruling out a contribution from stores containing $InsP_3$ receptors (Figures 1D and 1E). Finally, we applied different Ca^{2+} concentrations to the cytosol and measured the subsequent rhod-2 fluorescence increase (Figure 1F). The relationship yielded an apparent K_m of 16 ± 2 μM with a Hill coefficient of 1.2, which fit well with the reported affinity and cooperativity of the uniporter in isolated mitochondrial preparations [14, 15]. Collectively, these results demonstrate that the rhod-2 fluorescence is faithfully reporting the Ca^{2+} concentration within the mitochondrial matrix.

Patch-clamp recordings from mitoplasts have revealed that the uniporter is a Ca^{2+}-selective ion channel [11]. One important question is whether the uniporter is constitutively open or requires a ligand for activation. Ca^{2+}-uptake studies in isolated mitochondria suggest the presence of a slow, allosteric activation of the uniporter by Ca^{2+} [15]. On the other hand, whole mitoplast patch-clamp recodings reveal that the uniporter conducts Na^+ in the absence of cytoplasmic Ca^{2+}, indicating that Ca^{2+} is not needed for channel activity [11]. Consistent with a role for Ca^{2+} in activating the uniporter, the calmodulin antagonists calmidazolium and W-7 reduced mitochondrial Ca^{2+} uptake after stimulation with 20 μM Ca^{2+} (Figure 2A). Aggregate data are summarized in Figure 2B. Calmidazolium and W-7 still impaired mitochondrial Ca^{2+} uptake even when cytosolic Ca^{2+} was raised to 100 μM (data not shown). However, inhibition was always partial, and approximately 30%–40% of the response remained even in the presence of high concentrations of each inhibitor. Hence, activation of the mitochondrial Ca^{2+} uniporter in RBL cells is at least in part a Ca^{2+}-calmodulin-gated process. These results are in agreement with a previous study reporting that calmodulin antagonists inhibited the slow phase of Mn^{2+} flux through the uniporter [12]. Effects of calmodulin are often mediated by calmodulin-dependent protein kinase II (CaM kinase II). However, pretreatment with the CaM kinase II inhibitor KN-62 (10 μM; Figure 2B) or the broad protein kinase blocker staurosporine (2 μM; data not shown) failed to impair mitochondrial Ca^{2+} uptake.

In some cell types, cytosolic Ca^{2+} oscillations are propagated into mitochondria, resulting in a mitochondrial Ca^{2+} rise. However, mitochondrial Ca^{2+} then declines even though cytosolic Ca^{2+} continues to oscillate

*Correspondence: anant.parekh@physiol.ox.ac.uk

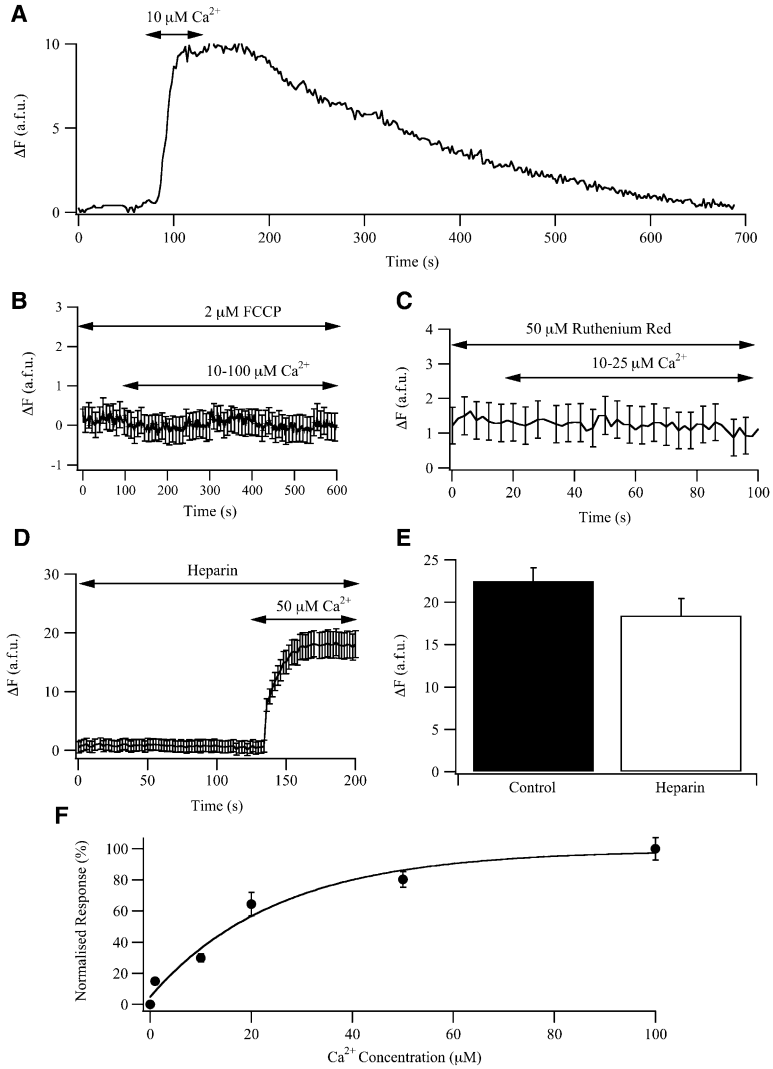

Figure 1. Rhod-2 Faithfully Reports Mitochondrial Ca^{2+} Signals

(A) After cell permeabilization with digitonin, a brief 10 μM Ca^{2+} application induced a rapid rise in rhod-2 fluorescence in digitonin-permeabilized cells (>100 cells).

(B) Digitonin-permeabilized cells were pretreated with 2 μM FCCP for 4 min before application of 10–100 μM Ca^{2+} (44 cells).

(C) Raising cytosolic Ca^{2+} failed to elicit an increase in rhod-2 fluorescence if cells were pre-exposed to 50 μM ruthenium red for 4 min (49 cells).

(D and E) Heparin (1 mg/ml) had no effect on the rise in rhod-2 fluorescence after challenge with 20 μM Ca^{2+} (40–63 cells for each point).

(F) Mitochondrial Ca^{2+} rise was measured after exposure to various cytoplasmic Ca^{2+} loads. Data are normalised to the peak response obtained in 100 μM Ca^{2+}, and each point represents between 57 and 126 cells. The relationship was fitted to a Hill equation. Aggregate data are presented as mean ± S.E.M.

[16, 17], leading to the suggestion that the uniporter might desensitize and hence curtail further mitochondrial Ca^{2+} influx. Such a mechanism would have significant impact on the spatiotemporal aspects of a cytosolic Ca^{2+} signal. To test this idea more directly, we compared the rate and extent of the mitochondrial Ca^{2+} rise after stimulation with a high concentration of cytoplasmic Ca^{2+} (100 μM) with and without a preceding brief Ca^{2+} pulse. Whereas a robust mitochondrial Ca^{2+} rise was seen in response to 100 μM Ca^{2+}, the response was dramatically reduced after a 60 s pre-pulse of 10 μM Ca^{2+} and subsequent perfusion with Ca^{2+}-free solution for several minutes (Figure 3A). We considered various explanations for this effect. Ca^{2+} uptake into mitochondria could depolarize the mitochondrial potential and reduce the driving force for further Ca^{2+} uptake. However, a brief pulse of Ca^{2+}, which was sufficient for triggering inactivation of the uptake mechanism, had no discernible effect on the mitochondrial membrane potential, whereas subsequent application of FCCP resulted in a sizeable depolarization (inset to Figure 3A). Ca^{2+} overload of mitochondria can trigger the opening of the large conductance permeability transition pore (PTP). PTP opening would be expected to clamp the potential at depolarized potentials and thus reduce the electrical driving force for Ca^{2+} influx into the matrix. Although we failed to observe a clear depolarization to Ca^{2+}, we nevertheless examined the effects of the PTP inhibitor cyclosporin A. Even after pretreatment with cyclosporin A, 100 μM Ca^{2+} was still unable to stimulate Ca^{2+} uptake when it was applied after the Ca^{2+} pre-pulse (Figure 3B). Finally, we considered the possibility that the activity of the mitochondrial Na^{+}-Ca^{2+} extrusion pathway might be enhanced after the Ca^{2+} pre-pulse. This could mask a potential Ca^{2+} rise induced by subsequent application of 100 μM Ca^{2+}. However, inhibition of the mitochondrial Na^{+}- Ca^{2+} exchanger with CGP37157 failed to rescue the response to 100 μM Ca^{2+} after a 20 μM Ca^{2+} pre-pulse (data not shown). Pozzan and colleagues [18] have suggested that the impaired mitochondrial Ca^{2+} uptake that was reported in a previous study [17] and which was attributed to desensitization of the uptake pathway instead reflected damage to rhod-2 and that this damage arose from the very high levels of laser illumination used. We therefore considered the possibility that the apparent inactivation of mitochondrial Ca^{2+} uptake was a consequence of either photobleaching or photodamage to rhod-2 or enhanced

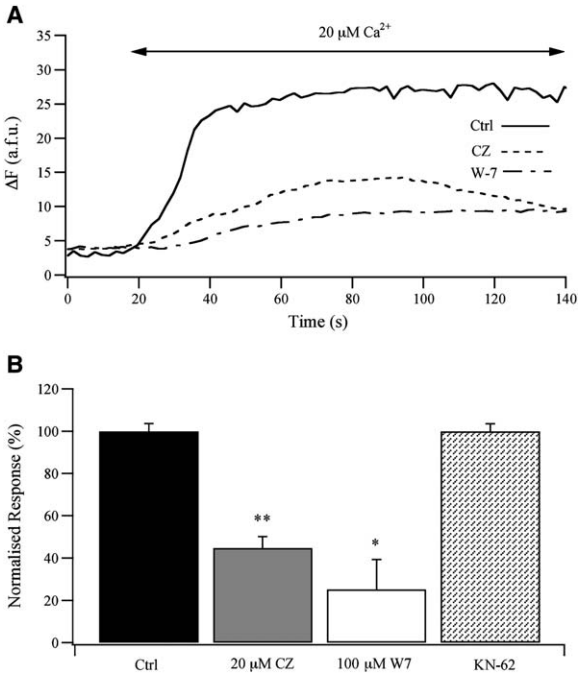

Figure 2. Ca^{2+}- Calmodulin Regulates the Uniporter

(A) Mitochondrial Ca^{2+} uptake after exposure to 20 μM cytosolic Ca^{2+} was significantly reduced by the calmodulin inhibitors calmidazolium (CZ; dashed line; 20 μM) and W-7 (dashed/dotted line; 100 μM).

(B) Aggregate data comparing the mitochondrial Ca^{2+} rise that followed challenge with 20 μM Ca^{2+} between control cells (Ctrl) and those pre-exposed for 15 min to calmidazolium, W-7, and 10 μM KN-62 (number of cells for each bar > 51). Data have been normalized to the peak response observed under control conditions. Aggregate data are presented as mean ± S.E.M.

removal of rhod-2 from the matrix. Two pieces of evidence militate against such a scenario in our study. First, although 100 μM Ca^{2+} failed to raise mitochondrial Ca^{2+} after a 20 μM Ca^{2+} pre-pulse, subsequent application of the Ca^{2+} ionophore ionomycin (which increases mitochondrial permeability to Ca^{2+}) resulted in a large rise in rhod-2 fluorescence (ΔF of 18.0 ± 1.8) that was similar in extent to that seen with 100 μM Ca^{2+} alone (i.e., in the absence of a Ca^{2+} pre-pulse; ΔF of 22.9 ± 1.6; $p > 0.2$). Hence, compartmentalized rhod-2 was still able to respond to rises in matrix Ca^{2+} concentration. Second, in some experiments, we applied 20 μM Ca^{2+} for 60 s but did so in the absence of rhod-2 excitation. Illumination began shortly before application of 100 μM Ca^{2+}, and the response was still significantly impaired (by 92% ± 5%), despite the much shorter image-acquisition time.

Collectively, these results suggest that the mitochondrial uniporter is inactivated by a cytoplasmic Ca^{2+} rise. Ca^{2+} inhibition was maintained even though the cytosolic Ca^{2+} load and mitochondrial Ca^{2+} had both returned to resting levels (Figure 3A). Hence, inactivation does not require a sustained cytosolic or mitochondrial Ca^{2+} rise. This hysteresis effect of Ca^{2+}-dependent inactivation, where a short Ca^{2+} rise induces long-lasting inhibition, is reminiscent of the hysteresis seen with CaM kinase II. However, pre-treatment with either calmidazolium or KN-62 prior to the 20 μM Ca^{2+} pre-pulse failed to rescue the response to a high Ca^{2+} challenge (Figure 3C). Furthermore, pretreatment with the broad protein kinase blocker staurosporine (2 μM) also failed to prevent the development of Ca^{2+}-dependent inactivation (data not shown).

To obtain the Ca^{2+} dependence of inactivation, we applied different concentrations of Ca^{2+} to the cytosol for 60 s, followed this with perfusion in Ca^{2+}-free solution for 5 min, and then measured the response after a challenge with 100 μM Ca^{2+}. Aggregate data are summarized in Figure 3D (filled squares). Ca^{2+} uptake fell monotonically with the rising amplitude of the preceding Ca^{2+} pre-pulse, and the IC_{50} for Ca^{2+} inhibition was approximately 15 μM.

Superimposed in Figure 3D is the corresponding mitochondrial Ca^{2+}-uptake curve (open squares). Strikingly, inspection of the activation and inactivation curves revealed that cytosolic Ca^{2+} concentration had a dual effect on mitochondrial Ca^{2+} uptake. Ca^{2+} initially increased mitochondrial Ca^{2+} uptake (in part because of Ca^{2+}-calmodulin activation of the uniporter), but higher Ca^{2+} then inactivated the uptake pathway. This is reminiscent of Ca^{2+}-dependent gating of $InsP_3$ receptors, although the latter is manifest at much lower cytosolic Ca^{2+} concentrations [19, 20]. Ca^{2+}-dependent positive and negative feedback on $InsP_3$ receptor activity has been used to explain the generation of cytosolic Ca^{2+} oscillations and the propagation of global Ca^{2+} waves. Ca^{2+}-dependent gating of mitochondrial Ca^{2+} uptake raises the intriguing possibility that mitochondrial Ca^{2+} dynamics might also contribute to the initiation or propagation of cytosolic Ca^{2+} waveforms.

The finding that the Ca^{2+} affinities for mitochondrial Ca^{2+} uptake and Ca^{2+}-dependent inactivation were similar (approximately 10–20 μM) implies that, in order for mitochondria to buffer cytosolic Ca^{2+} effectively, the rate of Ca^{2+} uptake should be faster than the rate of inactivation. The time constant of mitochondrial Ca^{2+} uptake (measured from experiments as in Figure 2A) was 7.9 ± 0.4 s in 10 μM Ca^{2+}, 5.1 ± 0.9 s in 20 μM Ca^{2+}, and 3.0 ± 1.7 s in 100 μM Ca^{2+}. To obtain the time-constant of Ca^{2+}-dependent inactivation, we applied a pre-pulse of 10 μM Ca^{2+} for different times, perfused the cytosol with Ca^{2+}-free solution for 6 min, and then applied a high concentration of Ca^{2+} (100 μM). As shown in Figure 3E, inactivation of the uptake pathway with Ca^{2+} occurred with a time constant of 16.0 ± 1.4 s, twice as slow as the rate of mitochondrial Ca^{2+} uptake at the same Ca^{2+} concentration (10 μM). The finding that elevated cytosolic Ca^{2+} levels for several seconds are needed to evoke clear inactivation of the mitochondrial uptake pathway suggests that cytosolic Ca^{2+} oscillations of short duration might be repetitively taken up by mitochondria with little inactivation of the uniporter. Indeed, in ventricular myocytes from neonatal rats, cytosolic Ca^{2+} oscillates rapidly with a frequency of approximately 1 Hz, and this is associated with rapid Ca^{2+} uptake by mitochondria in synchrony with the cytosolic Ca^{2+} oscillations [21].

The cytosolic Ca^{2+} concentrations that are required for half-maximal mitochondrial Ca^{2+} uptake and Ca^{2+}-dependent inactivation are approximately 10–20 μM, raising the question of whether such high Ca^{2+} concentrations are achieved after physiological stimulation. Mitochondria can be located close to Ca^{2+} release and Ca^{2+} influx channels and hence can be exposed to large

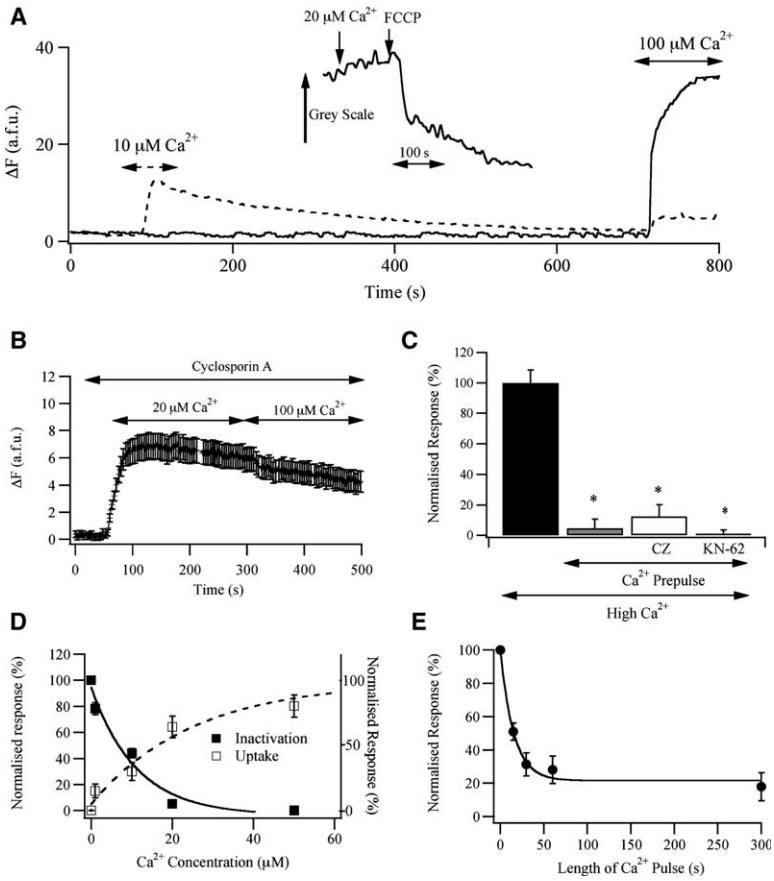

Figure 3. Ca^{2+}-Dependent Inactivation of the Uniporter

(A) The solid line depicts a control response to a 100 μM cytosolic Ca^{2+} pulse. The dashed line shows the response after a brief pre-pulse with 10 μM Ca^{2+}. The inset shows mitochondrial potential changes measured with JC-1.

(B) Inhibition of the PTP with cyclosporin (10 μM; pretreated for 15 min) fails to prevent the development of Ca^{2+}-dependent inactivation (27 cells).

(C) Pretreatment (15 min) with calmidazolium (20 μM) or KN-62 (10 μM) fails to rescue the inactivation seen upon application of 100 μM Ca^{2+} 6 min after a 60 s Ca^{2+} pre-pulse. Data have been normalized to the response to 100 μM Ca^{2+} in the absence of any pretreatment.

(D) Aggregate data from several experiments in which the cytosolic Ca^{2+} pre-pulse amplitude was varied are shown. Each point reflects between 28 and 49 cells. Superimposed is the Ca^{2+} dependence of mitochondrial Ca^{2+} uptake. The overlap of the two curves reveals the biphasic dependence of mitochondrial Ca^{2+} uptake on cytosolic Ca^{2+}. Both sets of data have been normalized to the response seen in 100 μM Ca^{2+} alone.

(E) Kinetics of development of Ca^{2+}-dependent inactivation. An initial Ca^{2+} pulse of 20 μM was applied for varying lengths of time (10–300 s). Cells were then perfused with Ca^{2+}-free intracellular solution plus 100 μM EGTA for 6 min before being subjected to the 100 μM Ca^{2+} test pulse. Each point represents 30–62 cells. Data are expressed as the percent response to the 100 μM Ca^{2+} test pulse in the absence of any pre-pulse.

Aggregate data are presented as mean ± S.E.M.

local Ca^{2+} signals [6]. Consistent with this, we found that Ca^{2+} release from the endoplasmic reticulum after exposure to the second messenger $InsP_3$ resulted in a rapid rise in mitochondrial Ca^{2+} uptake (Figure 4A) and that this occurred at a rate similar to that seen after stimulation with 10 μM Ca^{2+}. Hence, after $InsP_3$-dependent Ca^{2+} release, mitochondria are exposed to a local Ca^{2+} signal that is high enough to support both Ca^{2+}-dependent activation and inhibition of the uniporter. The mitochondrial Ca^{2+} rise after $InsP_3$ stimulation was transient and declined to a level slightly above the prestimulation value within 100 s (see below). Calculations reveal that, in order to be exposed to an approximately 10 μM Ca^{2+} microdomain spreading from an open ion channel, the Ca^{2+} sensor should be located tens to a few hundreds of nm from the Ca^{2+} source [22]. Using electron microscopy, we therefore looked for a close association between mitochondria and endoplasmic reticulum. As shown in Figures 4C and 4D, several mitochondria are located within 10–200 nm of endoplasmic reticulum, and this proximity would facilitate Ca^{2+} coupling between the two organelles. This is in good agreement with the findings from Rizzuto and colleagues, whose results showed that mitochondria are exposed to microdomains of elevated Ca^{2+} emanating from open $InsP_3$ receptors in the endoplasmic reticulum [6, 7, 23]. One important factor that helps determine the spatial extent of a Ca^{2+} microdomain from an open $InsP_3$ receptor is the rate of Ca^{2+} flux through the open channel. The nonmetabolizable $InsP_3$ analog inositol 2,4,5-trisphosphate is a partial agonist of $InsP_3$ receptors and releases Ca^{2+} at a rate 65% that of $InsP_3$ [24]. If mitochondria indeed respond to local Ca^{2+} signals, then the rate of rise of mitochondrial Ca^{2+} after Ca^{2+} release from $InsP_3$ receptors should be slower when inositol 2,4,5-trisphosphate rather than $InsP_3$ is the agonist. As shown in Figure 4A, this was indeed the case when maximally effective concentrations of inositol 2,4,5-trisphosphate and $InsP_3$ were used (see Figure 4B for aggregate data). Mitochondrial Ca^{2+} declined considerably more slowly when Ca^{2+} release was evoked by inositol 2,4,5-trisphosphate than was the case with $InsP_3$ (Figure 4A). Hence the transient nature of the $InsP_3$ response is in large part due to $InsP_3$ metabolism [25].

Concluding Remarks

The past few years have heralded a renaissance in our understanding of the role of mitochondria in regulation of intracellular Ca^{2+} signaling and subsequent cell responses, including cell death. Mitochondria take up Ca^{2+} from the cytosol via a Ca^{2+}-selective uniporter channel. We have found that mitochondrial Ca^{2+} uptake has a biphasic dependence on cytosolic Ca^{2+}. Uptake is facilitated by Ca^{2+}-calmodulin, whereas sustained cytosolic Ca^{2+} inactivates the pathway. The Ca^{2+} concentrations required for significant activation and inactivation

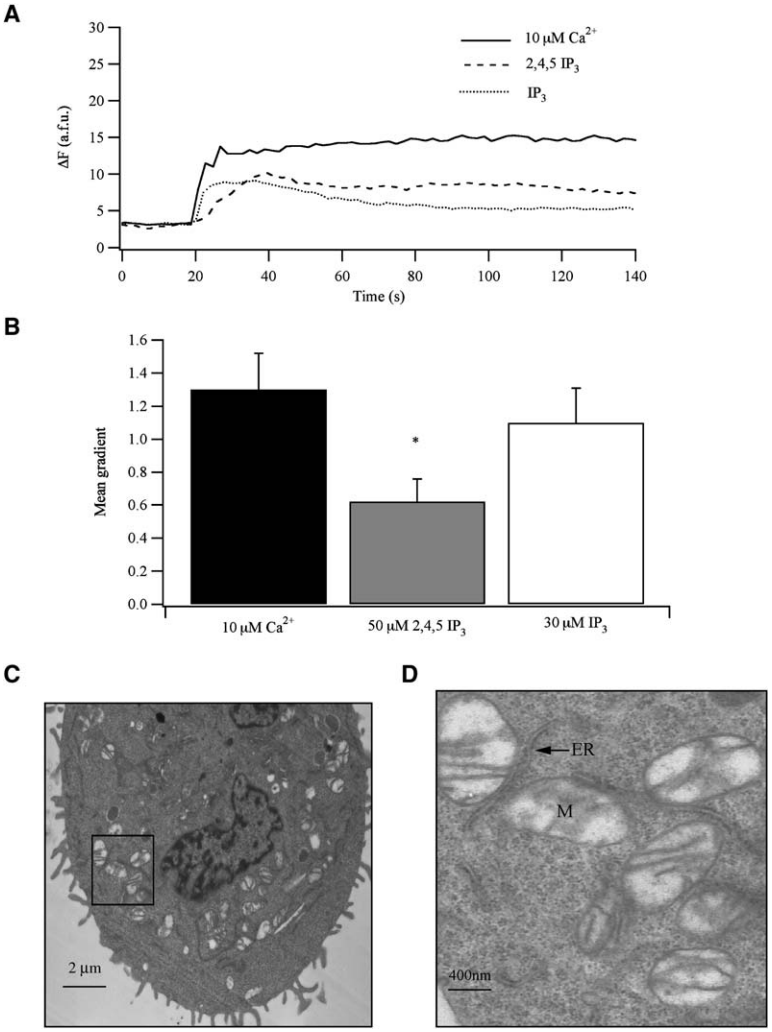

Figure 4. Mitochondria Sense Microdomains of Local Ca^{2+} Release

(A) The rate of mitochondrial Ca^{2+} uptake was compared for a 10 μM Ca^{2+} load (solid line), a pulse of 30 μM $InsP_3$ (dashed line), and a supramaximal dose of inositol 2,4,5-trisphosphate (50 μM; dotted line), which is a nonmetabolisable $InsP_3$ analog that is a partial agonist of $InsP_3$ receptors on the endoplasmic reticulum.

(B) Aggregate data from several experiments are summarized (number of cells > 35 for each bar).

(C) A transverse section through an RBL cell shows close proximity between a portion of the mitochondria and the endoplasmic reticulum.

(D) Magnification of boxed region shown in (C). ER and M denote endoplasmic reticulum and mitochondria, respectively.

Aggregate data are presented as mean ± S.E.M.

are reached after $InsP_3$-dependent Ca^{2+} release, suggesting that Ca^{2+}-dependent gating is likely to be of physiological relevance. Ca^{2+}-dependent inactivation of mitochondrial Ca^{2+} uptake means that mitochondria will not be able to remove Ca^{2+} from the cytosol effectively. Hence, mitochondria should not be considered simply as high-capacity passive buffers. Rather, the Ca^{2+} dependence imparts strong plasticity to mitochondrial buffering. This, coupled with the fact that they are motile, suggests that mitochondria are highly dynamic Ca^{2+}-controlling units whose properties, and hence influence on Ca^{2+} signaling, are strongly sculpted by Ca^{2+} itself.

Experimental Procedures

RBL-1 cells were cultured as described previously [25]. Mitochondria in RBL-1 cells were loaded with rhod-2 by incubation with Rhod-2 AM (2 μM) for 60 min in Ca^{2+} Ringer solution in the dark and then allowed to de-esterify in Ca^{2+} Ringer external solution for approximately ten minutes. Mitochondria were loaded with JC-1 after incubation for 15 min with 2 μM of the mitochondrial-membrane-potential-sensitive dye. Cytosolic rhod-2 was removed by permeabilization with 5 μM digitonin (present for 5 min) in an intracellular medium designed to energize the mitochondria (120 mM KCl, 10 mM NaCl, 2 mM KH_2PO_4, 10 mM HEPES, 1 mM $MgCl_2$, 1 mM succinic acid, 1 mM pyruvic acid, 50 μM EGTA, 2 mM Mg-ATP [pH 7.4] with KOH). Tracking the loss of cytosolic fura 2 (after loading by incubation for 40 min with 2 μM fura 2-AM) in real time after application of digitonin allowed the concentration and time of exposure to digitonin to be determined empirically. After permeabilization, cells were kept in the intracellular medium. Mitochondrial-Ca^{2+} imaging experiments were carried out at room temperature with the IMAGO charge-coupled-device camera-based system from TILL Photonics. Rhod-2 was excited at 540 nm, and emission at >560 nm was collected. JC-1 was excited at 490 nm. Data are presented as the relative increase in rhod-2 fluorescence above resting levels (ΔF, arbitrary fluorescent units [afu]). Images were acquired with Tillvision software and then analyzed with Igor Pro. Data are presented as means ± sem, and * and ** denote $p < 0.05$ and $p < 0.01$, respectively (unpaired t test).

Electron Microscopy

Cells growing on coverslips were fixed in 4% paraformaldehyde and 0.5% gluteraldehyde in 0.1 M phosphate buffer at pH 7.4 for 2 hr. The same phosphate buffer was used to wash and postfix the cells in 1% osmium tetroxide for 45 min. Cells were then washed in distilled water and stained with 2% aqueous uranyl acetate for 1 hr. Coverslips were dehydrated through a series of alcohols (30%, 50%, 70%, 90%, and 100%), inverted, and then embedded in Araldite resin. Once samples had polymerized, coverslips were peeled from the resin, leaving the cells embedded within the resin surface. Whole cells were serially sectioned at 70 nm, via a Reichert ultramicrotome, and collected on formvar-coated grid slots. Grids were poststained with 2% lead acetate and viewed in a Jeol 400 transmission electron microscope. Micrographs were then digitized.

Acknowledgments

This work was supported by a Medical Research Council program grant to A.B.P., and B.M. holds a British Heart Foundation studentship.

Received: June 8, 2006
Revised: June 29, 2006
Accepted: June 30, 2006
Published: August 21, 2006

References

1. Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: Dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. *4*, 517–529.
2. Duchen, M.D. (1999). Contributions of mitochondria to animal physiology: From homeostatic sensor to calcium signalling and cell death. J. Physiol. *516*, 1–17.
3. Rizzuto, R., Bernardi, P., and Pozzan, T. (2000). Mitochondria as all-round players of the calcium game. J. Physiol. *529*, 37–47.
4. Babcock, D., and Hille, B. (1998). Mitochondrial oversight of cellular Ca^{2+} signalling. Curr. Opin. Neurobiol. *8*, 398–404.
5. Gunter, T.E., Gunter, K.K., Sheu, S.S., and Gavin, C.E. (1994). Mitochondrial calcium transport: Physiological and pathological relevance. Am. J. Physiol. *267*, C313–C319.
6. Rizzuto, R., and Pozzan, T. (2006). Microdomains of intracellular calcium: Molecular determinants and functional consequences. Physiol. Rev. *86*, 369–408.
7. Rizzuto, R., Pinton, P., Carrington, W., Fay, F.S., Fogarty, K.E., Lifshitz, L.S., Tuft, R.A., and Pozzan, T. (1998). Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. Science *280*, 1763–1766.
8. Hajnoczky, G., Hager, R., and Thomas, A.P. (1999). Mitochondria suppress local feedback activation of inositol1,4,5-trisphosphate receptors by calcium. J. Biol. Chem. *274*, 14157–14162.
9. Duchen, M.R. (2000). Mitochondria and Ca^{2+} in cell physiology and pathophysiology. Cell Calcium *28*, 339–348.
10. Gilabert, J.-A., and Parekh, A.B. (2000). Respiring mitochondria determine the pattern of activation and inactivation of the store-operated Ca^{2+} current ICRAC. EMBO J. *19*, 6401–6407.
11. Kirichok, Y., Krapivinsky, G., and Clapham, D.E. (2004). The mitochondrial calcium uniporter is a highly selective ion channel. Nature *427*, 360–364.
12. Csordas, G., and Hajnoczky, G. (2003). Plasticity of mitochondrial calcium signaling. J. Biol. Chem. *278*, 42273–42282.
13. Boiter, E., Rea, R., and Duchen, M.D. (1999). Mitochondria exert a negative feedback on the propagation of intracellular calcium waves in rat cortical astrocytes. J. Cell Biol. *17*, 795–808.
14. Gunter, K.K., and Gunter, T.E. (1994). Transport of calcium by mitochondria. Journal of Bioenergetics and Biomembranes *26*, 471–485.
15. Gunter, T.E., and Pfeiffer, D.R. (1990). Mechanisms by which mitochondria transport calcium. Am. J. Physiol. *258*, C755–C786.
16. Maechler, P., Kennedy, E.D., Wang, H., and Wollheim, C.B. (1998). Desensitization of mitochondrial calcium and insulin secretion responses in the beta cell. J. Biol. Chem. *273*, 20770–20778.
17. Collins, T.J., Lipp, P., Berridge, M.J., and Bootman, M.D. (2001). Mitochondrial calcium uptake depends on the spatial and temporal profile of cytosolic calcium signals. J. Biol. Chem. *276*, 26411–26420.
18. Fillipin, L., Magalhaes, P.J., Di Benedetto, G., Colella, M., and Pozzan, T. (2003). Stable interactions between mitochondria and endoplasmic reticulum allow rapid accumulation of calcium in a subpopulation of mitochondria. J. Biol. Chem. *278*, 39224–39234.
19. Bezprozvanny, I., Watras, J., and Ehrlich, B.E. (1991). Bell-shaped calcium-response curves of Ins(1,4,5)P3- and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature *351*, 751–754.
20. Finch, E.A., Turner, T.J., and Goldin, S.M. (1991). Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. Science *252*, 443–446.
21. Robert, V., Gurlini, P., Tosello, V., Nagai, T., Miyawaki, A., Di Lisa, F., and Pozzan, T. (2001). Beat-to-beat oscillations of mitochondrial $[Ca^{2+}]$ in cardiac cells. EMBO J. *20*, 4998–5007.
22. Neher, E. (1998). Vesicle pools and Ca^{2+} microdomains: New tools for understanding their roles in neurotransmitter release. Neuron *20*, 389–399.
23. Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993). Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria. Science *262*, 744–747.
24. Marchant, J.S., Chang, Y.-T., Chung, S.-K., Irvine, R.F., and Taylor, C.W. (1997). Rapid kinetic measurements of $45Ca^{2+}$ mobilization reveal that Ins(2,4,5)P3 is a partial agonist at hepatic InsP3 receptors. Biochem. J. *321*, 573–576.
25. Parekh, A.B., Fleig, A., and Penner, R. (1997). The store-operated calcium current I_{CRAC}: Nonlinear activation by $InsP_3$ and dissociation from calcium release. Cell *89*, 973–981.